Cell Stem Cell
Previews

One Breath Closer to Making Engineered Tissues a Clinical Reality

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Reported recently in *Lancet*, Macchiarini and colleagues (2008) implanted a living tissue-engineered airway in a female patient. The restoration of the patient's quality of life testifies to this successful translation of benchtop to bedside studies and provides promise for the application of regenerative medicine strategies to other clinical disorders.

Paolo Macchiarini and coworkers recently used an engineered graft to replace a diseased portion of windpipe in a woman who had failed all previous treatments, and they succeeded in restoring breathing function to this patient (Macchiarini et al., 2008). Their strategy involved use of a biologic scaffold in the form of a donated human trachea that was pretreated to remove endogenous cells and immune-stimulatory proteins. This scaffold was seeded with two populations of patient-derived cells: cultured epithelial cells isolated by a small airway biopsy, and cartilage cells derived in vitro from bone marrow stem cells (BMSCs) obtained from a needle aspirate. The cell-seeded scaffold was placed in a custom bioreactor and subjected to hydrodynamic stimuli (similar to breathing) and enhanced nutrition such that the patient-derived cells integrated with the donated tracheal scaffold and produced living tissue. The surgery, in which a 5 cm section of the left bronchus was replaced with the tissue-engineered graft, has restored normal lung function in the patient. Further, follow-up biopsies indicate revascularization and incorporation of the graft with no evidence of rejection at 4 months after surgery. Based on this short-term success and their preclinical animal studies (Jungebluth et al., 2009; cited in Macchiarini et al., 2008), the scientists and surgeons involved remain cautiously optimistic regarding the long-term outcome for this patient, and enthusiastic about the potential implications of their work on the field of regenerative medicine. The authors are thoughtful in their presentation, providing context for their findings and demonstrating a methodical approach from laboratory to patient. More importantly, this study highlights the successful coordination of areas under intensive research in regenerative medicine, including investigation of cell sources, growth factors, scaffolds, and bioreactors (Figure 1).

It is unlikely that a single tissue-engineering blueprint can be developed that would successfully repair and replace all candidate tissues or organs, as the optimal approach will need to be tailored to the specific clinical demand, degree of injury, tissue complexity, and required function of the graft in each case, and will likely also reflect the timely availability of scaffolds and an appropriate cell source. The feature that distinguishes the current advance from previously reported human-implanted engineered tissues comprised of cellularized scaffolds (e.g., skin [Parenteau, 1999], cartilage [Ochi et al., 2004], and bladder [Atala et al., 2006]) is the incorporation of autologous native differentiated cells and laboratory-differentiated stem cells into a decellularized human-donor scaffold. Encouraging for the current trachea study, autologous endothelial cell progenitor cell-seeded human donor decellularized pulmonary valves have been reported to remain functional in two pediatric patients at 3.5 years follow-up (Cebotari et al., 2006). The overall impact of a strategy using allogeneic decellularized tissue scaffolds may

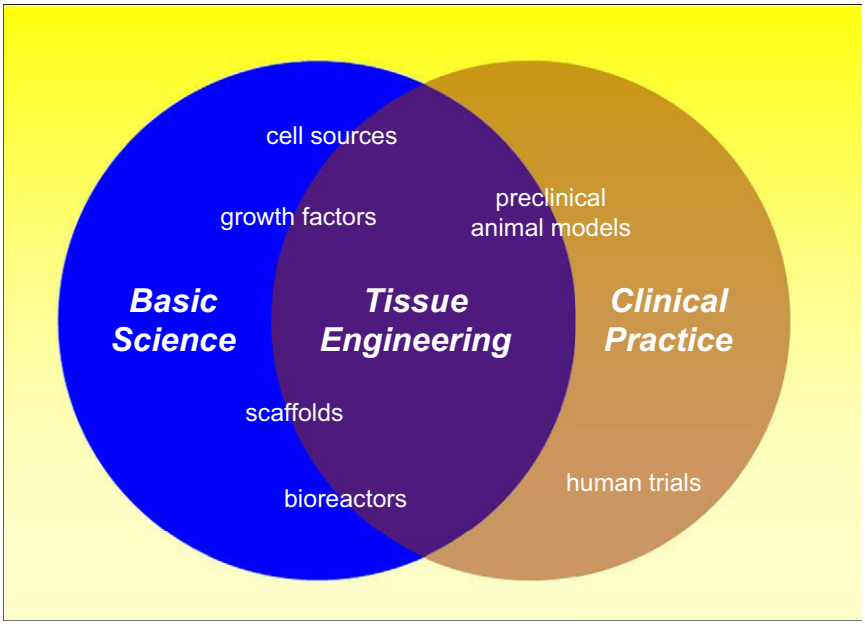

Figure 1. Venn Diagram Depicting Tissue Engineering at the Intersection of Basic Science and Clinical Practice, and Translation of Benchtop to Bedside Studies

ultimately depend on the prevalence of the clinical problem to be addressed. The reliance on human donor tissues predisposes this approach to issues of insufficient supply of healthy tissue of appropriate size, concerns of disease transmission, and regulations associated with tissue retrieval, banking, matching, and distribution. While the detergent protocol used for this work provides significant advantage with respect to tissue processing and matching, and the time frame for cell seeding was very clinically relevant, the hurdles of tissue availability, sizing, banking, and distribution, as well as the bioreactor treatment of the scaffolds, will still need to be addressed before widespread use of this type of tissue-engineering strategy can be considered.

In order to develop an engineered structure to restore a functional airway in the patient, the major requirements were to establish a nonimmunogenic graft that also exhibited functional mechanical properties. The decellularized, detergent-treated donor trachea fulfilled these criteria. It was a tubular scaffold that possessed the biochemical and structural properties of healthy trachea tissue, including hyaline cartilage rings to impart mechanical strength and flexibility. Use of a functional organ-donor scaffold can greatly reduce the coculture time required to seed cells prior to implantation, which was 4 days in this case. In contrast, tissue-engineering strategies using natural or synthetic material-derived scaffolds to grow tissues de novo typically require weeks to months to achieve acceptable levels of cell-elaborated tissue formation and function prior to implantation.

Engineering of tissues (particularly those that are in higher demand or require more complex structures) may be more readily pursued with a de novo tissue fabrication approach, in that "from scratch" engineering provides greater flexibility in design and construct dimensions, and no requirement for donor tissue. In this context, bioreactors that adopt a functional tissue-engineering paradigm of physiologic loading (Butler et al., 2000) have been used successfully to create functional tissue in culture. Deformational loading bioreactors, as an example, concomitantly present a physical stimuli and enhanced nutrient and growth factor transport to grow cartilage with native mechanical properties in vitro (Mauck et al., 2003). An alternative to these ex vivo approaches is the use of the in vivo environment as the incubator for tissue development. Biocompatible scaffolds can be created with tissue-specific functional mechanical properties such that they can be immediately implanted (with or without cells) into patients without preculture (Moutos et al., 2007). In this strategy, the burden on the scaffold and cells includes restoration of tissue biochemical composition and structure in addition to maintenance of functional mechanical properties of the graft. Optimal indications for ex vivo versus in vivo strategies may vary based on a number of patient-, technique-, and application-related factors.

The turnaround time between initial cell procurement and subsequent clinical implantation of the engineered tissue is also affected by the expediency of culture protocols aimed at cell expansion and commitment to or maintenance of a desired differentiated phenotype prior to scaffold seeding. A combination of native differentiated epithelial cells and BMSC-derived chondrocytes was used in the current application, and other groups have used, or hope to use, stem cells from adipose tissue, muscle, embryonic stem cells, and induced pluripotent stem cells (Takahashi et al., 2007) for tissue engineering in regenerative medicine. While the use of combinations of cell types may offer advantages with respect to regeneration of complex tissues and organs, use of a single, readily expandable cell source to generate a range of tissues or organs is also attractive on many levels. The most efficacious cell type in a given situation may depend on the strategy being pursued and the demands on the derived cells once implanted.

While researchers have been successful in guiding stem cells to express appropriate genes of a target differentiated cell type using various stimuli, the ability of stem cells to produce de novo tissue in the laboratory thus far appears modest at best. Therefore, the strategy of early implantation with mechanically functional scaffolds may be preferred for stem cell-based strategies. As preclinical animal studies from Macchiarini and coworkers (Jungebluth et al., 2009) indicate that growth factors from the decellularized trachea may promote angiogenesis and that cells from adjacent tissues can rapidly repopulate the graft, it is possible that preseeding of clinical grafts may be unnecessary. Whichever the case may be, strategies to optimize cell seeding and attachment, recruitment, differentiation, and synthetic activity via growth factors or gene therapy enhancement of scaffolds in conjunction with spatially varying scaffold designs hold tremendous promise for regenerative medicine.

The investigative team should be applauded for their significant achievement. In their own words, "the findings suggest that autologous cells combined with appropriate biomaterials might provide successful treatment for patients with serious clinical disorders" (Macchiarini et al., 2008). The encouraging results in restoration of quality of life of the patient serve as tangible motivation for the maturing field of regenerative medicine and reaffirm that fundamental basic science studies can be successfully translated to the clinic.

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